REMARKS

The Claimed Invention

The presently claimed invention is directed to the covalent labeling of immobilized poly(amino acids) with reactive dipyrromethaneboron difluoride dyes for the detection and analysis of poly(amino acids). This method is particularly useful when poly(amino acids) have been separated on a gel and then transferred to a membrane or alternatively when apatemers are immobilized and used to capture poly(amino acids). Due to the covalent labeling of all the immobilized proteins this method is particularly useful in conjunction with a specific label that has a detectably different label from the dipyrromethaneboron difluoride dyes.

The Pending Claims

Prior to entry of the attached amendments, Claims 1-70 are pending. Claims 1-8 and 71 are directed to a method for labeling immobilized poly(amino acids). Claims 9-13 and 72 are directed to a method for labeling poly(amino acids) bound to aptamers. Claims 14-19 are directed to a method for labeling immobilized poly(amino acids) in an array. Claims 20-56 are directed to a method for detecting immobilized poly(amino acids). Claims 57-70 are directed to kits for detection of poly(amino acids) immobilized on a solid surface. Claims 73-80 are directed to a method for detecting immobilized poly(amino acids) that have first been separated by gel electrophoresis.

The Office Action

Claims 57-70 are provisionally withdrawn.

Claims 1-57 stand rejected under 35 U.S.C. 112 second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 9-56 stand rejected under 35 U.S.C. 103(a) as being unpatentable over the admitted prior art as set forth at pages 1 through 5 of the instant application.

Amendments

Claims 1,2, 4, 6, 8-11, 14-16, 20, 33, and 36 have been amended.

Claims 1, 9, 14, and 20 have been amended to remove "L-Rx" and replace with "maleimide, amine-reactive group". Support can be found in Claim 1 as filed and on page 16 lines 20-21.

Claim 1 has been amended to remove the text in brackets.

Claims 1, 9, 14, and 20 have been amended to clearly indicate that the R^1 through R^7 substituents can be substituted or unsubstituted. Support can be found on page 15 lines 14-20.

Claims 1, 9, 14, and 20 have been amended to indicate that at least one of R^1 through R^7 is a maleimide or amine-reactive group. Support can be found in Claim 1 as filed.

Claim 2 has been amended to remove the language "for dipyrromethaneboron difluoride dye, Rx".

Claims 4 and 13 have been amended to remove the language "made of materials that are".

Claim 5 has been cancelled.

Claims 6, 11, 16 and 36 have been amended to clarify that the concentration of the dye in the labeling mixture is "about 5 micromolar to about 20 micromolar". Support can be found on page 17 lines 4-6.

Claim 6 has been amended to change the claim dependency from Claim 2 to Claim 1.

Claim 7 has been cancelled.

Claim 8 has been amended to change the claim dependency from Claim 7 to Claim 1.

Claims 10 and 15 have been amended to clarify that the succinimidyl ester of a carboxylic acid is an amine reactive group and to provide antecedent basis. Supoprt can be found in Claim 19 and 10 as filed and on page 16 lines 20-22.

Claim 11 has been amended to change the dependency from Claim 9 to Claim 10.

Claim 11 has been amended to remove "combined" to provided antecedent basis for "the labeling mixture".

Claim 12 has been cancelled.

Claim 17 has been cancelled.

Claim 18 has been cancelled.

Claim 34 has been cancelled.

Claim 35 has been cancelled.

Claim 36 has been amended to remove the language "wherein said labeled poly(amino acids) are illuminated for five seconds or less".

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Claim 46 has been amended to add the word "of" after "capable", as suggested by the Examiner.

New Claims 71-80 have been added. Support can be found throughout the specification and claims as filed. In particular support for Claims 71, 72 and 80 can be found in Claims 21-31. Support for Claims 73-79 can be found in Claims 1-20 and 32.

Applicants believe that no new matter has been added by any of these amendments and the Examiner is respectfully requested to enter them.

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RESPONSE TO THE RESTRICTION REQUIREMENT

In the response that follows, the Examiner's Election/Restriction of the Applicant's claimed invention is provided in full text, as identified by indented small bold print, followed by the Applicants response.

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. **Claims 1-56, drawn to methods of fluorescently labeling and detecting immobilized polyamino acids, classified in class 530, subclass 409; class 436, subclass 546; class 435, subclasses 7.5 and 7.9.**

II. **Claims 57-70, drawn to kits comprised of a functionalized dipyrromethaneboron difluoride dye in combination with a labeled specific binding pair member, classified in class 530, subclass 391.3; class 436, subclass 806.**

The inventions are distinct, each from the other because of the following reasons:

Inventions I and II are unrelated. Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different modes of operation, different functions, or different effects (MPEP § 806.04, MPEP § 808.01). In the instant case, the method of labeling poly(amino acids) of claim 1 (invention I), which is directed to the reaction between an immobilized poly(amino acid) and a functionalized dye, does not require the use of the kit of claim 57 (invention II) which is comprised of the combination of a labeled specific binding pair member and a functionalized dye. Further, the two inventions involve different patentability considerations. A patentability determination for invention I would involve the determination of the novelty and unobviousness of *a combination of process steps* including a separation of poly(amino acids) by gel electrophoresis, immobilization of the separated poly(amino acids) followed by covalent bonding of the poly(amino acids) to a functionalized dye. A patentability determination for invention II, on the other hand, would involve the determination of the novelty and unobviousness of the combination of functionalized dye and labeled specific binding pair member *independent of any particular method of use of the combination.*

Because these inventions are distinct for the reasons given above and have acquired a separate status in the art because of their recognized divergent subject matter requiring different fields of search and different patentability considerations, restriction for examination purposes as indicated is proper.

During a telephone conversation with Koren Anderson on February 26, 2004 a provisional election was made with traverse to prosecute the invention of invention I, claims 1-56. Affirmation of this election must be made by applicant in replying to this Office action. Claims 57-70 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

Applicants affirm that, as required by CFR 1.143, Applicants have provisionally elected group I (Claim 1-56), with traverse. Claims 57-70 have been provisionally withdrawn.

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RESPONSE TO THE REJECTIONS

In the response that follows, the Examiner's individual rejections are provided in full text, as identified by indented small bold print, followed by the Applicant's response.

35 U.S.C. 112, 2nd ¶ Rejection

Claims 1-56 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicants have amended or cancelled specific claims to overcome the Examiners rejection of Claims 1-56. Applicants believe that the Claims are now definite and particularly point out and distinctly claim the subject matter, which they believe is their invention. The Examiner is respectfully requested to withdraw this rejection of Claims 1-56

a) In claim 1, page 56, lines 18-22, the meaning of the bracketed words is unclear.

Applicants have amended Claim 1 to remove the text in brackets. In addition, Claim 1 along with Claims 9, 14 and 20 have been amended to clarify that each of the R^1 through R^7 substituents can be substituted or unsubstituted. Applicants believe that this amendment overcomes the rejection and respectfully request that it be withdrawn.

b) In claim 1, it is unclear whether the term "such that the dipyrromethaneboron difluoride dye has an absorption maximum between 495 nm and 640 nm" is meant to indicate that all of the recited dyes have an absorption maximum in the stated range or whether only a subset of the recited dyes has an absorption maximum in the stated range.

Table 2 and 3 list a number of the reactive dyes that are envisioned by the present application. Most of the dyes fall within the range of an absorption maximum between 495 nm and 640 nm. However, not all of the dyes have this property. Thus, Claims 1, 9, 14, and 20 have been amended to remove this unnecessary limitation. Applicants respectfully request that this rejection of these claims be withdrawn.

c) In claim 1, step d., it is unclear what functional moiety/moieties on the poly(amino acids) are involved in the formation of "a covalent bond" with the "reactive group" of the dye.

Claims 1, 9, 14, and 20 have been amended to clarify that at least one of R^1 through R^7 is a reactive group that is a maleimide or an amine-reactive group (See, Page 15 line 26 and

page 16 lines 20-24). Maleimide reacts selectively with thiol groups and amine-reactive groups react selectively with amine containing groups. Thus, these chemically reactive dye molecules react selectively and form a covalent bond with either thiol groups or amine groups on the poly(amino acids). It is understood that the choice of the reactive group on the present dye molecule dictates the functional group on the poly(amino acid) that the dye forms a covalent bond with. Applicants respectfully request that this rejection be withdrawn.

d) In claim 4, the plural term "materials that are poly(vinylidene difluoride)" is inconsistent with the fact that "poly(vinylidene difluoride)" is a single chemical entity.

Claims 4 and 13 have been amended to remove the language "materials that are poly(vinylidene difluoride)" to clarify that poly(vinylidene difluoride) is a single material, which is a solid support. Applicants respectfully request that this rejection be withdrawn.

e) In claim 5, it is unclear whether the combined molecular weight of all the poly(amino acids) is 500 to 200,000 Daltons or whether each poly(amino acid) has the stated molecular weight. See also, claim 34.

Claim 5 and other similar claims referring to the weight of the poly(amino acids) have been cancelled. Applicants respectfully request that this rejection be withdrawn.

f) For claim 11, there is no antecedent basis in claim 9 for the term "the combined labeling mixture".

Claim 11 has been amended to remove "combined" so that the claim reads "the labeling mixture"; Claim 9 contains antecedent basis for "the labeling mixture". Applicants respectfully request that this rejection of Claim 11 be withdrawn.

g) In claim 46, the word --of-- should appear after "capable".

Claim 46 has been amended to add the word "of" as suggested by the Examiner. Applicants respectfully request that this rejection of Claim 46 be withdrawn.

35 U.S.C. 103(a) Rejection

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Claims 9-56 are rejected under 35 U.S.C. 103(a) as being unpatentable over the admitted prior art as set forth at pages 1 through 5 of the instant specification.

Applicants respectfully traverse this rejection because the cited prior art in the background section of the present application do not make obvious the presently claimed invention. Specifically, the use of the present dyes to covalently label immobilized proteins is not obvious from the prior art based on the known properties of the dye. The Examiner is requested to withdraw this rejection of Claims 9-56.

The subject matter of claims 9-56 is directed to the fluorescent labeling of immobilized poly(amino acids) by the covalent attachment of well known dipyrromethene boron difluoride dyes and the subsequent detection of the label.

As described in the instant specification, the technique of covalently labeling poly(amino acids) with fluorescent dyes followed by detecting of a fluorescent optical response is well known in the art. The poly(amino acids) can be immobilized on conventional supports as well as being attached to immobilized aptamers (page 1, line 20 -page 2, line 7).

Given the fact that the dipyrromethene boron difluoride compounds of the instant claims are admittedly well known fluorescent dyes which are useful to label proteins (poly(amino acids)), it would be obvious to substitute these dyes as equivalents for the fluorescent dyes used in the prior art processes of labeling immobilized poly(amino acids), as claimed, with the expectation of obtaining a similarly useful labeling process with the known advantages accruing to the use of dipyrromethene boron difluorides. See in particular, the specification, page 4, line 28 -page 5, line 10 for a discussion of the desirable features of the dipyrromethene difluoride dyes of the instant claims and page 14 of Haugland, Handbook of Fluorescent Probes and Research Chemicals, 6th edition (cited on form PTO-1449) for a discussion of the use of these dyes to label peptides (poly(amino acids)).

Applicants respectfully assert that it is not obvious from the prior art that using known dipyrromethene boron difluoride dyes for the labeling and detection of immobilized poly(amino acids) would result in equivalent results compared to known methods using other reactive dyes.

There are three important points that relate to the non-obviousness of the invention; 1) the unexpected disappearance of the quenching phenomenon, 2) the long felt need in the art for a better reactive dye to label and detect the poly(amino acids), and finally 3) the duration of the time that the dyes and the method for immobilizing poly(amino acids) have been known prior to the present invention. These three points, when combined, demonstrate that the present invention is not obvious in view of the cited references. Furthermore, no one else recognized the value of combining the reactive dipyrromethene boron difluoride dyes with a method for immobilizing, labeling and detecting poly(amino acids).

Typically, when a protein is labeled with more than one molecule of dye, quenching of the florescent signal is often observed (This is detailed on page 14 of Haugland, Handbook of Fluorescent Probes and Research Chemicals, 6th edition). This is a significant disadvantage for developing a method to label proteins over a wide range of concentrations resulting in a useful dynamic range wherein a bright fluorescent signal is necessary. Thus, based on the known properties of the reactive dye, the disappearance of the quenching phenomenon was completely unexpected when labeling immobilized proteins. This is because the prior art taught that labeling proteins with a high concentration of dye would likely result in quenching.

Thus, based on the teaching of the prior art it is not obvious that immobilizing the proteins would alleviate this quenching effect and therefore allow the present reactive dipyrromethene boron difluoride dyes to be useful in the present methods. In fact, this was a totally unexpected result. It is important to note that if the quenching phenomenon had not been overcome that the present dye would not be ideal for the present method and would certainly not have resulted in a commercial product.

Despite the fact that other reactive dyes have been used to label immobilized proteins they all suffer from different drawbacks. These include, sensitivity to pH, low quantum yield, low photostability, limited linear range due to quenching, non-compatibility with laser-based gel scanners, and loss of signal from drying of stained membrane. Thus, as described in the background section of the present application, at the time of the present invention there was a long felt need in the art for a method using a reactive dye to label and detect poly(amino acids) that did not suffer from these disadvantages. The use of the present dye overcame the quenching phenomenon while not producing other properties that would contribute to any other drawbacks of an ideal dye for labeling immobilized poly(amino acids).

While reactive dipyrromethene boron difluoride dyes were previously known and immobilizing proteins was also known, no one combined these two aspects to arrive at the presently claimed invention. This combination unexpectedly solved a problem in the art by overcoming the known disadvantages of other utilized reactive dyes, as listed above. The Applicants cannot speculate as to why this is, but there is no teaching in the art cited that would motivate one of skill in the art to use reactive dipyrromethene boron difluoride dyes in the present method. In fact, the cited art would appear to teach away from the currently claimed method based on the teaching that the use of reactive dipyrromethene boron difluoride dyes often results in quenching when more than one molecule of dye is attached to a protein. The art teaches that using lower concentrations of the dye, not feasible in the present method, or using

different versions of the dye, also not part of the present invention, can reduce this quenching phenomenon.

The background section of the present application contains a discussion of known protein counterstains for immobilized proteins. These counterstains fall into two categories, immunological based and non-protein based. The immunological method employs an antibody that is selective for a desired epitope, wherein the antibody or intended target is visualized by the covalent attachment of a detectable label to the antibody. The non-protein method uses colorimetric and fluorescent dyes that either associate covalently or non-covalently with the immobilized proteins. The fluorescent counterstains that associate non-covalently with the immobilized proteins are advantageous over the colorimetric because the dynamic range of the fluorescent stains is larger, however they do not provide a permanent record of the immobilized protein due to the non-covalent association. In addition, some of these counterstains interfere with additional analysis and must be completely removed prior to such analysis.

The reactive counter stains that provide a permanent record by covalently binding to the immobilized proteins include fluorescamine, FITC, DTAF and dansyl chloride. The use of the present reactive dyes to label immobilized proteins is advantageous over the known reactive counter stains for a number of reasons. However, one advantage in particular allows for the feasibility of this counterstain as a commercial product, and that is the dynamic range of the counterstain. This is important because it not only allows for a low detection limit but also for a detection limit over an order of magnitude, See Table 3.

The dynamic range is achieved by using a relatively high concentration of dye. However, the known properties of the present reactive dye teach away from the use of a large molar excess of reactive dye. This is because the present dyes usually show significant fluorescence quenching when attached to proteins, "particularly when proteins are labeled with a large molar excess of the reactive bipyrromethaneboron difluoride dyes" (page 5 lines 10-14). due to quenching of the fluorescent signal. The Applicants have unexpectedly found that labeling immobilized proteins with the present reactive dyes do not result in quenching, this allows for the dynamic range necessary for identification of proteins.

Applicants respectfully assert that the presently claimed invention is not made obvious by the cited references. The Examiner is respectfully requested to withdraw this rejection of Claims 9-56.

The features of the dependent claims are either specifically described by the references or constitute obvious variations in parameters which are routinely modified in the art (e.g. conventional concentrations of dyes of claim 11; time of illumination of labeled poly(amino acids) of claim 36) and which have not been described as critical to the practice of the invention. For example, for the use of a dipyrromethene boron difluoride succinimidyl ester of a carboxylic acid as recited in claim 10, see page 14 of Haugland, Handbook of Fluorescent Probes and Research Chemicals, 6th edition, "BODIPY Succinimidyl Esters" (cited on form PTO-1449).

Applicants respectfully disagree that the dependent claims are not obvious variations of parameters routinely modified in the art. One such limitation is the use of a labeled antibody to detect a subset of proteins while simultaneous detecting the total proteins of the sample with the present dyes (See, Claim 8 for example). The simultaneous detection of immobilized total proteins of a sample and a subset within the total has not previously been demonstrated. The cited references in no way teach this method or make obvious to one of skill in the art that staining all the proteins with one "color" and then using different "color" labeled antibody to detect a select set of proteins can be utilized simultaneously with no wash steps. See Examples 1 – 2 and 3-15 for detailed methods of present claimed dichromatic detection of total proteins and a subset of proteins.

Applicants respectfully assert that the dependent claims are not made obvious by the cited references and the Examiner is respectfully requested to withdraw this rejection.

CONCLUSION

In view of the above amendments and remarks, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (541) 335-0203.

Respectfully submitted,

Date: July 2, 2004

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